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13. ABSTRACT *(Maximum 200 words)*

Obligately-marine fungi grew in artificial sea water with single hydrocarbons as their sole source of organic carbon and energy. The unsaturated compound 1,14-tetradecadiene and the methyl-branched compound pristane were used by several of the fungi. None of these fungi used aromatic hydrocarbons as their sole source of carbon. Two isolates, *Corollospora lacera* and *C. maritima*, were tested for growth on mixtures of hydrocarbons. Both cometabolized several aromatics while growing on glucose. *C. maritima* also cometabolized acenaphthalene, phenanthrane and dibenzothiophene while growing on hexadecane but not while growing on glucose. Aromatic hydrocarbons inhibited growth on glucose, and aromatic hydrocarbons were not cooxidized with glucose as growth substrate. The principal fatty acids of these organisms are 16:0, 18:0, 18:1n9 and 18:2n6. Of 11 isolates examined, one, a *Lulworthia* sp., contained a mitochondrial plasmid. Preliminary evidence suggests that four of five fungi examined form microbodies when the fungi are grown on hydrocarbons but not when they are grown on glucose.

14. SUBJECT TERMS
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FINAL REPORT

Grant #: N00014-01-J-1748

PRINCIPAL INVESTIGATOR: Dr. Joseph J. Cooney

INSTITUTION: University of Massachusetts Boston

GRANT TITLE Marine Fungi as Novel Catalysts for Bioremediation of Oil Spills

AWARD PERIOD: 1 April 1991 - 31 March 1995

OBJECTIVE: To determine the potential of obligately-marine fungi for degrading hydrocarbons in oil spills.

APPROACH: Pure cultures of marine fungi known to be associated with beach sand and with submerged wood were examined for the ability to grow on pure hydrocarbons and on mixtures of hydrocarbons. Using these fungi, a series of questions was posed experimentally in order to determine their usefulness as agents of bioremediation and to determine mechanisms by which they use hydrocarbons.

ACCOMPLISHMENTS: 1. Thirteen obligately-marine fungi were screened for the ability to grow in artificial sea water with a single hydrocarbon as their sole source of organic carbon. Each organism grew slowly on one or more hydrocarbons. Each had its own pattern of substrates utilized, but *n*-alkanes of intermediate chain length were the best growth substrates. The unsaturated compound 1,14-tetradecadiene and the methyl-branched compound pristane were used by several organisms. None of the fungi used aromatic hydrocarbons as their sole source of carbon and energy.

2. Two isolates, *Corollospora lacera* and *C. maritima*, were tested for growth on mixtures of hydrocarbons. Neither organism grew on mixtures of 10 or 11 hydrocarbons, but both cometabolized several aromatics from the 10-hydrocarbon mixture when growing on glucose. *C. maritima* also cometabolized acenaphthalene, phenanthrene and dibenzothiophene while growing on hexadecane, but not while growing on glucose with no alkane present. Each of the aromatic compounds except the least soluble, pyrene, inhibited fungal growth on glucose to an extent reflecting their relative solubilities.

3. Naphthalene, methylnaphthalene, acenephthalene and a mixture of all three caused rapid and significant leakage of potassium from *C. maritima.* Methylnaphthalene. The three-hydrocarbon mixture also caused significant protein leakage.

4. The principal fatty acids of these fungi, determined by gas chromatography, are 16:0, 18:0, 18:1n9 and 18:2n6.

5. Of 21 isolates examined, one, a *Lulworthia* sp., contains a mitochondrial plasmid which is approximately 14kb in size. The plasmid, designated pQB, is linear as shown by

the results of both UV-nicking experiments and digestion with exonuclease III and lambda exonuclease. The plasmid waspresent in DNA extracted from mitochondria, and it banded with mitochondrial DNA when nuclear and mitochondrial DNA were separated on a cesium chloride gradient. Thes results suggest that the plasmid is mitochondrial in origin. Southern analysis showed that the plasmid is not homologous with nuclear or mitochondrial genomic DNA, and therefore replicates autonomously.

6. The enzymes involved in initial oxidations of *n*-alkanes are constitutive in some of these fungi and inducible in others.

CONCLUSIONS: These obligately-marine fungi can grow on a wide variety of hydrocarbons, although they do so slowly. In the presence of an oxidizable substrate such as hexadecane or glucose the range of hydrocarbons used was expanded to include normally-recalcitrant aromatics and the one sulfur-containing aromatic compound tested. Some aromatic compounds, however, caused leakage of cellular components. These organisms could be valuable supplements to microbial seeds used to clean up spilled oils. The cellular fatty acids are typical of fungi. The organisms appear to form microbodies when they are grown on hydrocarbons but not when they are grown on glucose. The presence of a plasmid in one fungus suggests that these organisms can be manipulated genetically to enhance their hydrocarbon-using abilities.

SIGNIFICANCE: Marine fungi have considerable potential as participants in oil spill cleanup, particularly for expanding the range of hydrocarbons degraded.

PATENT INFORMATION: None

AWARD UNFORMATION:

1.In 1998 the Waksman Award for teaching and research was given to the principal investigator by the Society for Industrial Microbiology.

2. In 1992 the principal investigator was elected President-elect of the Society for Industrial Microbiology. He served as President in 1993.

M.M. Doolittle, a graduate student, was awarded a predoctoral Fulbright to work in Canada.

PUBLICATIONS AND ABSTRACTS:

1. Cooney, J.J. Microbial ecology and hydrocarbon degradation. Presented at a U.S.E.P. A. symposium held in Cincinnati, OH, published in J. Clean Technol. Environ. Sci. 2: 65- 71 (1992).

2. Cooney, J.J., M.M Doolittle, S. Wuertz, M.E. Miller and C. Baisden. Marine fungi: potential catalysts for bioremediation of oil spills. Abstr. Annu. Mtg. Amer. Soc.Microbiol., New Orleans, LA (1992).

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5. Cooney, J.J., S. Wuertz, M.M. Doolittle, M.E. Miller. K. Henry and D. Ricca. Marine Fungi: novel catalysts for bioremediation of oil spills. Trends in Microbiol. P 639-642 (1993).

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7. Baisden, C.M. and J.J. Cooney. Isolation and characterization of a plasmid from the Marine fungus *Lulworthia* sp. Abstr. Annu. Mtg. Amer. Soc. Microbiol. (1995).

8. Cooney, J.J., C.M. Baisden, D.M. Ricca and S.H. Flint. Degradation of hydrocarbons by marine fungi. VI Internat. Marine Mycology Symp., Portsmouth, England.

9. Baisden, C.M. and J.J. Cooney. Screening marine fungi for plasmids and Characterization of a mitochondrial plasmid in a *Lulworthia* sp. Mycologia 88: 350-357 (1996).

10. Flint, S.H. and J.J. Cooney. Interactions of alkane and aromatic hydrocarbons with marine fungi. In preparation.

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